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Saccharification of Lignocellulosic Biomass under Mild Condition Using Ionic Liquid

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Biomass is expected to be an alternative resources to fossil resources. In this study, the development of biomass conversion method into the valuable chemical, reducing sugar, was examined. For the conversion, thermochemical technology was focused on for its advantage of short reaction time, and ionic liquid was focused as reagent to overcome an unpreferable disadvantage of thermochemical technology, which is, the low selectivity. Cedar and crystalline cellulose were pretreated with ionic liquid for a reforming into desirable precursors of reducing sugar. Especially when they were pretreated by 1-Ethyl-3-methylimidazolium methylphosphonate at 150 ℃ for 1 h, pretreatment worked effectively by decreasing the crystallinity of samples. Pretreated cedar and crystalline cellulose were converted into reducing sugar under the hydrothermal condition, respectively, by 39 C-% and 90 C-%. Recovery of ionic liquid was also examined. When cedar was used as a material, lignin was dissolved into ionic liquid through pretreatment, which was undesirable because of their difficult separation. When crystalline cellulose was used as a material, 98.3 % of ionic liquid was recovered after the conversion with the highest yield of reducing sugar (90 C-%).

Introduction

Biomass has a potential to be an alternative resource to crude oil. However, it has the problems of high collecting cost and limited available amount. Therefore, to make biorefinery feasible, its superiority to oil refinery needs to be considered. Crude oil usage is mainly categorized into energy use and production of chemicals. For the production of chemicals, crude oil is usually converted into ethylene or propylene once, and then converted into chemicals. These chemicals include oxygen containing chemicals such as ethylene oxide or acetaldehyde. To produce these oxygen containing chemicals from ethylene or propylene, an oxidation process is required. However, an oxidation process generally has the problem of very low selectivity. On the other hand, biomass already contains oxygen in its structure. Although the oxygen is considered as disadvantage in the point of energy use because it decreases the heat of combustion, it could be considered as huge superiority of biomass for the production of chemicals. **Figure 1** shows the values of O/C plotted against the H/C based on the elemental composition of chemicals. Contrary to the crude oil derivatives, biomass derivatives require dehydration, hydration or reduction which possibly enable the selectivity to be 100 %. In addition, biomass-derived products can be used as energy resources in the end of their use through combustion. Thus, the use of biomass as the material for the production of chemicals seems to be more reasonable than the direct use as the energy resource. Biomass mainly consists of three components; cellulose, hemicellulose and lignin. About woody biomass, the main component is cellulose, which is the most abundant natural resource on this planet. This means the development of the new conversion method of cellulose is essential for the conversion of biomass into valuable chemicals. In this study, the effective conversion method of biomass or cellulose into reducing sugar, the product obtainable by the hydrolysis of cellulose, was investigated. Biomass is normally converted into reducing sugar by thermochemical technology or biochemical technology. Biochemical technology has huge advantage of high selectivity. This is the indispensable advantage for the utilization of biomass as an alternative resource. However, biochemical

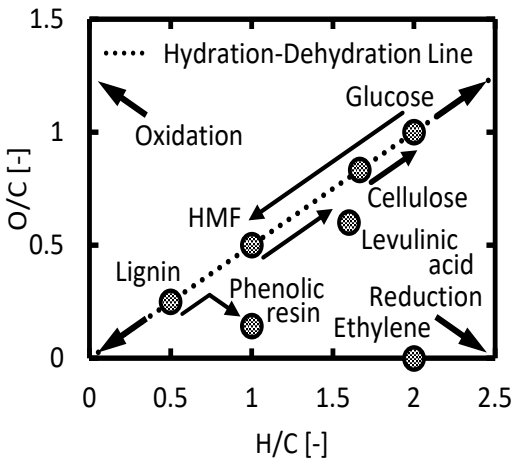

Fig. 1 Conversion pathways of biomass components and ethylene into chemicals

technology also has the disadvantages of long reaction time and high cost due to the expensive enzymes, the need of sewage treatment and complicated control of reactor. Consequently, the development of thermochemical technology for the effective conversion of biomass was investigated. The advantage of thermochemical technology is that it does not require long reaction time. On the other hand, there are some disadvantages as well such as low selectivity and huge energy consumption. To overcome these problems, the unique character of ionic liquid was focused on. Ionic liquid is the salt which is able to exist as liquid under relatively low temperature (Forsyth *et al*., 2004). Although the melting point of salts is generally high, it is possible to be lowered by making the radius of cation and anion bigger and weakening the electrostatic force between them. Despite the weakened electrostatic force between ions, the force is still strong enough to have features such as non-volatility, incombustibility and ionic conductivity. For its unique character, ionic liquid is used for many applications such as lubricant, solvent or electrolyte, and there have been many studies on ionic liquid recently, e.g., Phillips *et al*. (2004), Liu *et al*. (2012) and Barthel *et al*. (2006). Swatloski *et al*. (2002) and Sievers *et al*. (2009) reported that, among the many types of ionic liquid, the types containing the imidazole group are effective on solubilization of cellulose and biomass breaking the rigid structure of cellulose. For example, 1-Ethyl-3-methylimidazolium bromide ([EMIM]Br) breaks the rigid structure of cellulose and solubilizes biomass. Cellulose solubilized by this ionic liquid is known to be recovered by adding another liquid which has no ability of dissolving cellulose (Vo *et al*., 2012). Because of this unique characteristic, cellulose is supposed to be easily uncrystallized and separated from ionic liquid. Another example is 1-Ethyl-3-methylimidazolium methylphosphonate ([EMIM]P). [EMIM]P has high ability of breaking the rigid structure of cellulose, and it changes cellulose to water-soluble cellulose. Uju *et al*. (2012) reported that solubilization of cellulose enables some reagents such as enzymes or acids to attack the active cites of cellulose more effectively. For example, Dadi *et al*. (2006) reported that cellulose could be converted into reducing sugar in the yield of 90 % by using ionic liquid. However, in this efficient method, cellulose is converted by biochemical technology and it requires reaction time of 50 h. Another example using ionic liquid, via thermochemical technology, was reported by Li *et al*. (2007), which enables cellulose conversion into reducing sugar reach 77 % by the treatment of 9 h at 100 ℃. However, this method introduces acidic catalyst in the process of hydrolysis, which requires the additional separation process. In this study, thus, we pretreated biomass or cellulose with ionic liquid at first to solubilize them and to make desirable precursors highly selective for reducing sugar, and then degraded obtained precursors to the reducing sugar under the hydrothermal condition without using any reagent except for ionic liquid and water.

1. Experimental

All the experimental procedures explained in this section are briefly summarized in **Figure 2**.

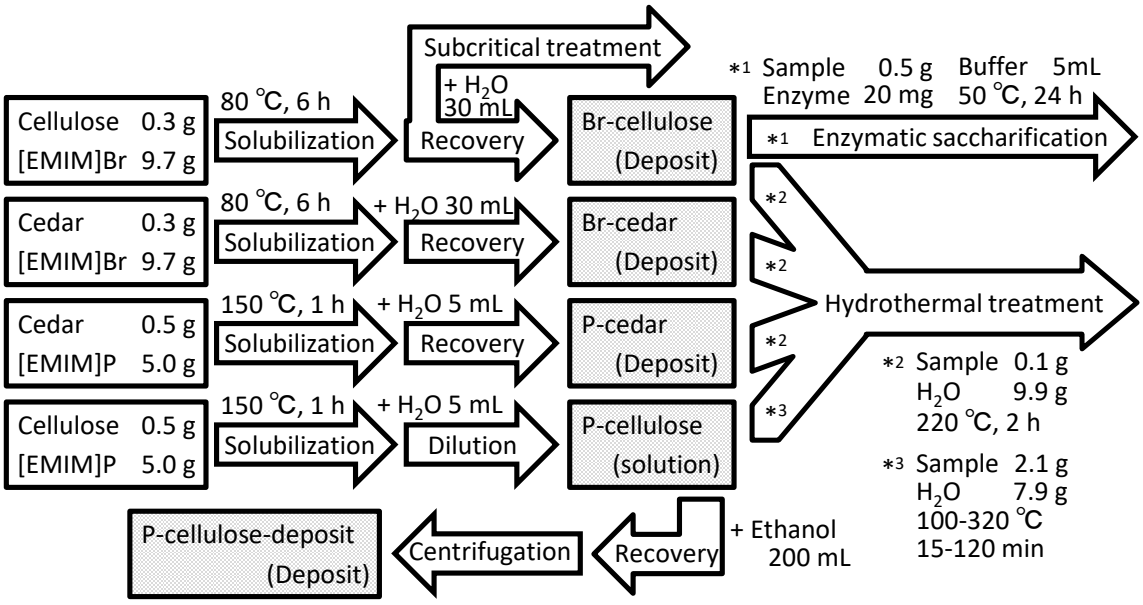

Fig. 2 Reaction flowchart (*1 and *2 were conducted using crystalline cellulose or amorphous cellulose as well)

Table 1 Ultimate analyses of samples used

	C [wt%]	H [wt%]	O (diff.) [wt%]	H/C [-]
Crystalline Cellulose	42.95	6.20	50.85	0.14
Amorphous Cellulose	43.37	6.12	50.52	0.14
Cedar	46.87	4.84	48.28	0.10

1.1 Materials

For the efficient saccharification of cellulose, biomass and crystalline cellulose were pretreated with ionic liquid. [EMIM]Br or [EMIM]P was used as ionic liquid and cedar (cryptomeria japonica) was used as the biomass sample. [EMIM]Br was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan) and [EMIM]P was purchased from KANTO CHEMICAL CO, INC (Tokyo, Janapn). Cedar was purchased from Kobo Mokuyo-Daiku (Tokyo, Japan) and pulverized using crusher (AS ONE Corporation) to particles of under 500 μm. Crystalline cellulose and amorphous cellulose were obtained from Kao Corporation (Tokyo, Japan). The ultimate analyses of pulverized cedar, crystalline cellulose and amorphous cellulose are listed in **Table 1**.

1.2 Pretreatment

The pretreatments were performed by using glass bottle with an internal volume of 50 cm^3. When [EMIM]Br was used as the ionic liquid, 0.3 g of cedar or crystalline cellulose and 9.7 g of [EMIM]Br were mixed in a glass bottle and put in a water bath preheated to 80 ℃ for 6 h under stirring. Immediately after heating, the products were cooled, 30 mL of pure water was added for the deposition of the solute, and the mixture was filtered by suction. The deposit was rinsed with pure water, dried in vacuo, and then analyzed by CHNS elemental analysis, thermogravimetric(TG) analysis and X-ray diffraction(XRD). The deposit is denoted as Br-cellulose or Br-cedar hereafter in this article. When [EMIM]P was used as the ionic liquid, 0.5 g of cedar or crystalline cellulose and 5.0 g of [EMIM]P were mixed in a glass bottle and put in an oil bath preheated to 150 ℃ for 1 h under stirring. Immediately after heating, the products were cooled and 5 mL of pure water was added. For cedar sample, the products were filtered by suction and rinsed with pure water. The residue is donated as P-cedar hereafter in this article. For the cellulose sample, after adding 5 mL of water, 200 mL of ethanol was added for the recovery because crystalline cellulose was converted into water-soluble components by [EMIM]P, and then the deposit was separated by centrifuge. The deposit and the solubilized crystalline cellulose without the addition of ethanol are denoted as P-cellulose-deposit and P-cellulose hereafter in this article. The solid samples, P-cedar and P-cellulose-deposit, were dried in vacuo, and then analyzed by CHNS elemental analysis, TG analysis and XRD.

1.3 Enzymatic Saccharification

To investigate whether the pretreatment with ionic liquid works effectively, enzymatic sacchrification was conducted on Br-cellulose, crystalline cellulose and amorphous cellulose. *Aspergillus* was chosen for enzyme. 0.5 g of cellulose sample (Br-cellulose, crystalline cellulose or amorphous cellulose), 20 mg of enzyme and 5 mL of citrate buffer solution (0.1 mol/L, pH = 5.0) were mixed in 30 cm^3 glass bottle and heated at 50 ℃ for 24 h in a water bath under stirring. The products were filtered by suction after the reaction and filtrates were diluted with pure water for the analysis by high performance liquid chromatography (HPLC).

1.4 Recovery of Solubilized Crystalline Cellulose by [EMIM]Br with Subcritical Water

The direct degradation of solubilized crystalline cellulose by [EMIM]Br was examined using subcritical water for the recovery instead. Solubilized cellulose by [EMIM]Br was prepared as the section 1.2. Instead of adding 30 mL of water, 30 mL of dimethyl sulfoxide (DMSO) was added to lower the viscosity of the solution for the flow reaction process. The sample was collided with subcritical water at 250℃ under the pressure of 20MPa. The flow rate of the supplied sample or pure water was 1.5 mL/min or 0.5 mL/min, respectively. The products contained dispersed particles, which were separated by centrifuge. The supernatant liquid was diluted with pure water by 100 mL and analyzed by HPLC.

1.5 Hydrothermal Degradation

The hydrothermal treatments were performed by using Swagelok (316 stainless steel) batch reactor with an internal volume of 30 cm^3 or 10 cm^3. 0.1 g of Br-cedar, P-cedar or Br-cellulose and 9.9 g of pure water were mixed in a sealed batch reactor and put in an oil bath preheated to 220 ℃ for 2 h. The reactor was cooled in a water bath after the reaction and then the products were filtered by suction. Filtrates were diluted with pure water by 100 mL and analyzed by 3,5-dinitrosalicylic acid (DNS) method. For comparison, experiments with raw materials (cedar, crystalline cellulose or amorphous cellulose) were conducted according to the same method. Because the recovery of P-cellulose-deposit requires plenty amounts of ethanol, the hydrothermal treatment on crystalline cellulose pretreated with [EMIM]P was conducted using solubilized sample. 2.1 g of P-cellulose (which consist of 1.0 g of [EMIM]P, 1.0 g of pure water and 0.10 g of cellulose) and 7.9 g of water were mixed in a sealed batch reactor and put in an oil bath preheated to 100 - 320 ℃ for 15 - 120

min. The cooling process, filtration process and analysis were conducted according to the same method as the other samples.

1.6 Analyses of Products

Ultimate analysis of the samples was performed using a CHNS elemental analyzer (BEL Japan, Inc., ECS4010). The analysis of pyrolysis profile of solid sample was performed using a TG analyzer (Shimadzu, TGA-50). Crystallinity of samples was measured by XRD (Rigaku Corporation). For the organic acid analysis, an aqueous solution containing 951 mg/L of *p*-toluene sulfonic acid, 4185 mg/L of Bis-Tris, and 29 mg/L of ethylenediamietetraacetic acid was used as the eluent, and it was fed at 0.8 mL/min to the HPLC equipped with a sulfonated polystyrene gel column (Shim-pack SCR-102H) and an electric conductivity detector (Shimadzu, CDD-6A). The yield of each component of organic acids estimated from the above measurements was represented on the basis of dry and ash-free samples. The yield of reducing sugar was calculated through the analysis by DNS method. DNS reagent was prepared by dissolving 4 g of NaOH, 1.25 g of dinitrosalicylic acid and 75 g of poptassium sodium tartrate into 400 mL of pure water. The mixture of the reagent and sample, 2 mL of each, was heated with boiling water for 10 min, cooled with iced water for 2 min, and then 3 mL of pure water was added. Absorbance for 540 nm of ultraviolet was measured using UV-Visible spectrophotometer (Shimadzu, UVmini-1240) for the yield calculation. The yield of reducing sugar was calculated by HPLC as well. An solution consists of 70 wt% of acetonitrile and 30 wt% of pure water was used as the eluent, and it was fed at 0.7 mL/min to the HPLC equipped with a sulfonated styrene divinyl benzene copolymer column (Shodex, RSpak DC-613) and a charged aerosol detector (Dionex, Corona).

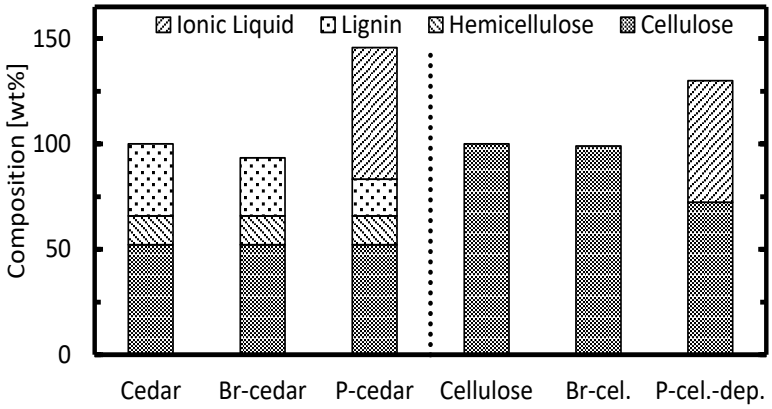

Fig. 3 Compositions of raw materials and pretreated samples

2. Results and Discussion

2.1 Pretreatment of Cedar

After the pretreatment of cedar with [EMIM]Br, 93.4 wt% of cedar was recovered as the deposit (Br-cedar). The compositions of the residue is shown in **Figure 3**. There was some weight loss through the pretreatment. This loss was considered as lignin-derived component because the hydrothermal degradation on filtrates was conducted and no cellulose-derived or hemicellulose-derived

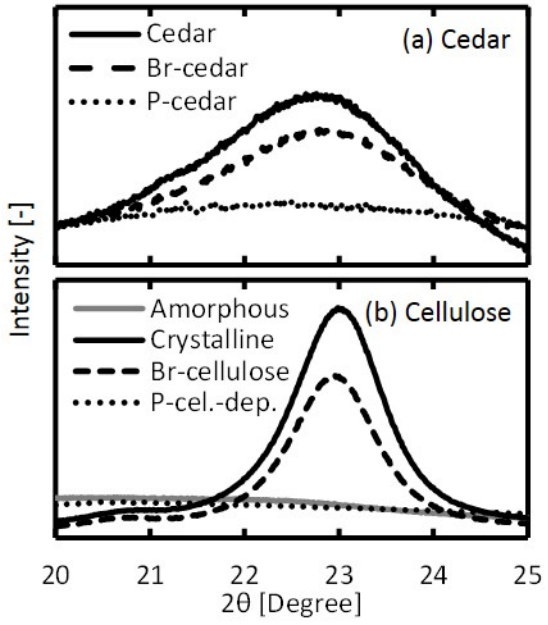

Fig. 4 XRD patterns of samples;
(a) Cedar, (b) Cellulose

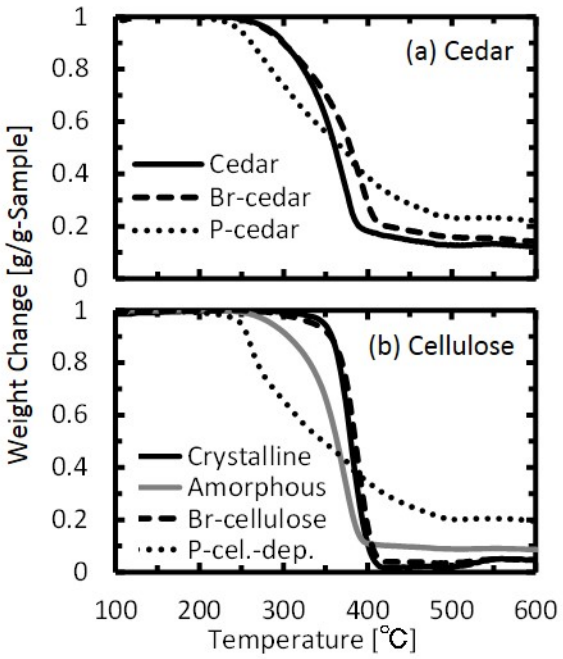

Fig. 5 TG curves of samples;
(a) Cedar, (b) Cellulose

Table 2 Elemental compositions of samples

	C	H	O (diff.) [wt%]	N	P
Br-cellulose	44.10	6.10	49.80	-	-
P-cellulose-deposit	38.16	7.13	42.83	5.64	6.24
Br-cedar	49.82	4.34	45.79	0.05	-
P-cedar	40.80	6.02	42.25	5.19	5.74

component was produced. Through the pretreatment with [EMIM]P, cedar was converted into the slurry-like components. The dry residue, P-cedar, was recovered by 145.8 wt % on the basis of the amount of cedar used. According to the analysis by CHNS elemental analysis and TG analysis, it was clarified that 83.3 wt% out of 145.8 wt% derived from cedar and rest of 62.5 wt% derived from [EMIM]P. To clarify the component of solubilized 16.7 wt%, the filtrates were treated under hydrothermal condition. Through the reaction, no cellulose-derived or hemicellulose-derived component was produced. According to this fact, the conversion into P-cedar was calculated as there were no decreases of cellulose and hemicellulose, and only lignin was lost as soluble component by the pretreatment. **Figure 4 (a)** and **Figure 5 (a)** show the XRD patterns, and the TG curves of the samples and the products. **Table 2** shows the elemental compositions of Br-cedar and P-cedar, where the values P were calculated according to the assumption that the values N derive from [EMIM]P. [EMIM]P broke into the cellulose making strong hydrogen bond to cellulose and it was impossible to be extracted from the sample by rinsing with pure water. On the other hand, [EMIM]Br was completely extracted into the liquid phase, which is very attractive in the economic point of view because ionic liquid is quite expensive and should be recovered. However, about the decrease of crystallinity, [EMIM]P was far more effective than [EMIM]Br. This is probably because, although [EMIM]Br broke the structure of cellulose and solubilized once, cellulose was re-crystallized during recovery process. Contrary to this, [EMIM]P broke the structure and kept being captured, preventing re-crystallization of cellulose, which led to the better decrease of crystallinity. Considering the purpose of the pretreatment with ionic liquid was to convert the raw materials into more degradative precursors, [EMIM]P seems to satisfy this better. Furthermore, the TG curves confirmed this by showing the lower decomposition temperature for P-cedar.

2.2 Hydrothermal Degradation of Pretreated Cedar

The main products through the hydrothermal treatment were reducing sugar and organic acids. **Figure 6** shows the carbon conversions into them on the basis of raw materials. The organic acids consist of formic and acetic acid for raw cedar, and succinic, glycolic and formic acid for pretreated cedar. The residue after the treatment was less for both pretreated cedar than raw sample cedar because of the loss of lignin during pretreatment, or because of the improved reactivity of samples through the pretreatment. Although there were some loss of lignin through the pretreatment, the yield of organic acids had increased, which indicates lignin surely had improved its reactivity according to the reports that lignin produces organic acids by hydrothermal treatment. However, about reducing sugar, Br-cedar produced less of it than raw sample. This was probably because cellulose in Br-cedar was stuck in lignin more rigidly than the raw material after the process of deposition. P-cedar increased the yield of reducing sugar as well as that of organic acids. The capture of [EMIM]P in its structure led to the decrease of crystallinity, and it seems to be one of the biggest factor for this increase, with the yield reaching 39 C-%. It is now revealed that ionic liquid changes the structure of cedar, however, there is a problem of recovery. As mentioned in the previous section, there were some amounts of lignin extracted to ionic liquid. The separation of lignin from ionic liquid is very difficult, and it causes huge loss of

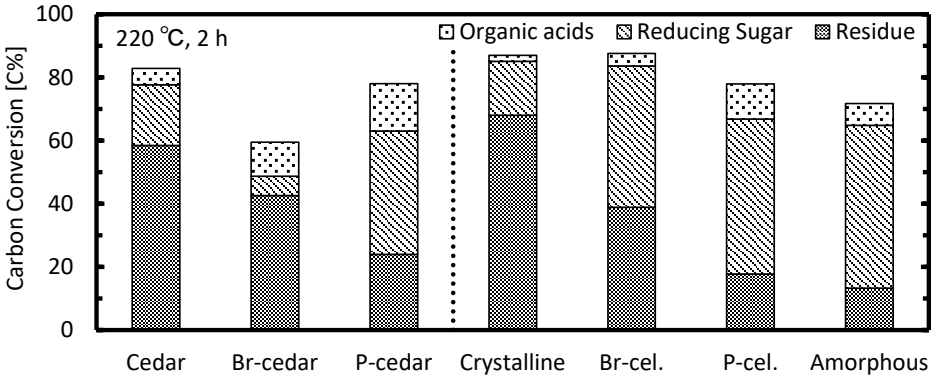

Fig. 6 Carbon conversions after hydrothermal treatment (on the basis of raw materials)

ionic liquid. The high value of ionic liquid thus makes this process infeasible.

2.3 Pretreatment of Crystalline Cellulose

Because of the recovery issue of ionic liquid when cedar was used as the sample, conversion of cellulose was examined next. There are some established methods for the separation of cellulose from biomass (e.g., Li *et al*., 2009), so the refinery from biomass is still feasible. After the pretreatment of crystalline cellulose using [EMIM]Br at 80 ℃ for 6 h, no solid cellulose was recognized in the sample before adding pure water. Then, by adding pure water, 99.0 wt% of cellulose was recovered as the deposit (Br-cellulose), without capturing [EMIM]Br. This fact that almost all cellulose was recovered after the pretreatment would be the confirmation for the hypothesis that [EMIM]Br did not extract any cellulose from cedar by the pretreatment (in the section 2.1). **Figure 3**, **Figure 4 (b)** and **Figure 5 (b)** show the compositions, the XRD patterns, and the TG curves of the samples, the products and also amorphous cellulose for comparison. **Table 2** shows the elemental compositions of Br-cellulose. All the analyses showed quite similar tendencies to cedar's case. Crystallinity of crystalline cellulose decreased by about 30 % through the pretreatment with [EMIM]Br judging from the XRD patterns. However, this decrease is not big considering that crystalline cellulose was completely solubilized once, so cellulose seemed to be recrystallized in the process of recovery. When the pretreatment with [EMIM]Br was conducted for 1 h or 3 h at 80℃, the crystallinity decreased, respectively, by 0 % and 22 %. Judging from the decreases between 1 h and 3 h, and between 3 h and 6 h, no decrease was expected for treatments longer than 6 h. In addition, all the samples obtained by [EMIM]Br treatment at 120℃ for 1 h, 3 h and 6 h showed similar XRD patterns and TG curves to those of obtained through 80 ℃ treatments, thus, the suitable condition for the [EMIM]Br pretreatment on crystalline cellulose was determined as 80 ℃, 6 h. Different from the pretreatment with [EMIM]Br, crystalline cellulose pretreated at 150 ℃ for 1 h with [EMIM]P was solubilized completely and then never recovered by adding pure water. Because when the pretreatment with [EMIM]P was conducted at 130 ℃, the mixed sample of crystalline cellulose and [EMIM]P was converted into gelatinous component and coagulated by adding pure water, the pretreatment temperature was determined as 150 ℃. Much ethanol was added to extract cellulose from the solution instead of pure water. The recovered deposit is donated as P-cellulose-deposit in this article, and the yield of the deposit was 130.0 wt%. This means some amounts of [EMIM]P was recovered together with cellulose. It was clarified by CHNS elemental analysis and TG analysis that 72.4 % of cellulose was recovered as P-cellulose-deposit and the rest of 57.6 % derived from [EMIM]P(**Figure 3**). The XRD pattern and TG curve of P-cellulose-deposit are shown in **Figure 4 (b)** and **Figure 5 (b)**, respectively. The elemental composition of P-cellulose-deposit is listed in **Table 2**. The crystallinity index of crystalline cellulose was very high comparing with cedar (about 5 times). Crystalline cellulose was uncrystallized very well and the thermal degradability increased by the pretreatment with [EMIM]P. Considering these results, P-cellulose-deposit seems to have improved its reactivity. However, the loss of cellulose during the pretreatment was not negligible. Besides, in the point of separation, solubilized cellulose was not necessarily separated from ionic liquid as the deposit before the following reactions because P-cellulose-deposit contained much ionic liquid to be separated later anyway. Thus, the following hydrothermal reaction was not conducted on P-cellulose-deposit but on P-cellulose, which is, the solubilized cellulose.

2.4 Enzymatic Saccharification of Br-cellulose

To confirm that crystalline cellulose was reformed to more degradative precursor, the enzymatic saccharification was conducted using crystalline cellulose, amorphous cellulose and Br-cellulose as samples. For the enzyme, *Aspergillus* was chosen. After the treatment at 50 ℃ for 24 h, 8.2 C-% of crystalline cellulose, 14.8 C-% of amorphous cellulose and 15.6 C-% of Br-cellulose was converted into reducing sugar. As expected, cellulose increased its reactivity by the pretreatment. Crystallinity is assumed to be one of the biggest factors to effect on saccharification, however, Br-cellulose produced more glucose than amorphous cellulose. This means any other factor but cristallinity had bigger effect when *Aspergillus* was used as an enzyme. Although 24 h of saccharification time is usually not long enough for enzymatic saccharification, the purpose of this treatment was to confirm that crystalline cellulose became more degradative by the pretreatment, which was achieved.

2.5 Recovery of Br-cellulose with Subcritical Water

Br-cellulose was obtained by adding pure water to the solubilized crystalline cellulose with [EMIM]Br. However, the following process, saccharification, would be conducted by adding more water and the heating. In that case, the recovery of the solubilized crystalline cellulose with subcritical water possibly has the potential of direct saccharification. This recovery process was conducted using flow reaction system, by the solubilized cellulose being collided with the subcritical water. Because the viscosity of the

solution was too high for the flow reaction system, DMSO was added to lower the viscosity of the solution before the treatment. At first, the effect of DMSO on the solution was examined by adding it at two different timings. One of them was adding DMSO to [EMIM]Br before the pretreatment and the other was adding DMSO to cellulose solution after the pretreatment. 98 % of crystalline cellulose was solubilized when DMSO was added after the pretreatment while 22 % was solubilized when DMSO was added before. This means DMSO had no effect on the deposition of solubilized cellulose but had the huge effect on the solubilization process. Because crystalline cellulose needed to be solubilized for the treatment with subcritical water, DMSO was added after the pretreatment as the preparation for the next process, and reducing sugar was obtained by 23.5 C-% finally. Although this yield was higher than that obtained by enzymatic saccharification, it was not high enough to replace conventional process yet. In addition, DMSO was added in this process, which makes the separation more complicated. Thus, it was concluded that this direct degradation process was not preferable.

2.6 Hydrothermal Degradation of Pretreated Crystalline Cellulose

To confirm that [EMIM]P would not be spoiled by heat during the hydrothermal degradation, [EMIM]P was heated to higher temperature and confirmed that it starts degrading around 400 ℃. To examine the effect of crystallinity to the yield of reducing sugar, the hydrothermal degradation was conducted at 220 ℃ on crystalline and amorphous cellulose prior to the pretreated samples, and the result is shown in **Figure 6**. From the result, it is obvious that crystallinity had huge effect on the production of reducing sugar. Then, the hydrothermal degradation on the pretreated samples were conducted. Crystalline cellulose pretreated with both types of ionic liquid decreased its crystallinity and produced more reducing sugar than crystalline cellulose as shown in **Figure 6**. P-cellulose was converted into reducing sugar by almost the same yield as amorphous cellulose, reaching 50 C-%. Br-cellulose produced more reducing sugar than crystalline cellulose, however, it was not uncrystallized enough to reach the yield from amorphous cellulose or P-cellulose. Next, the optimum reaction condition for hydrothermal treatment was sought. At first, the reaction temperature for the hydrothermal treatment was changed, and the result is shown in **Figure 7**. Although Br-cellulose was converted into reducing sugar better than crystalline cellulose, the temperature degradation began at was almost the same. Furthermore, the yield was not as high as that from amorphous cellulose. However, crystalline cellulose pretreated with [EMIM]P increased the yield of reducing sugar significantly. The change in the degradation temperature was also remarkable, and the optimum hydrothermal temperature for saccharification was lowered to 150 ℃, reaching the yield of 90 C-%. This yield was about the same as enzymatic saccharification reported by Dadi *et al*. (2006) through the treatment of 50 h, and higher than the acidic saccharification reported by Li *et al*. (2006). The yield started decreasing as the temperature increased higher than 150 ℃. The products started producing residue at higher temperature than 150 ℃, which led to the decrease of the yield. Although it was obvious that crystallinity had huge effect on the production of reducing sugar, this high yield of [EMIM]P treatment cannot be explained only by crystallinity. Consequently, how [EMIM]P effects on crystalline cellulose or saccharides was investigated. At first, how glucose reacts to [EMIM]P through the treatment was examined. 0.1 g of glucose and 1.0 g of [EMIM]P were mixed in a glass bottle and put in an oil bath preheated to 150 ℃ for 1 h under stirring. Immediately after heating, the products were cooled, mixed with 8.9 g of pure water, and again heated at 150 ℃ for 2h. The product was analyzed by the DNS method and HPLC. According to the DNS analyses, the amount of reducing sugar detected was only 15.2 % of initial amount. Different from that, the amount of reducing sugar detected by HPLC was even less, 4.3 %. For the confirmation, [EMIM]P was analyzed by the DNS method and it was revealed that [EMIM]P had no reduction ability. These facts indicate that equipped column for HPLC was not able to separate [EMIM]P and glucose in this pretreated sample, which did not happen to just the mixture of untreated [EMIM]P and glucose. In addition, it was also confirmed that there were no glucose degradants in the product such as 5-hydroxymethylfurfural or levulinic acid. In summary, what possibly happened was, glucose was polymerized or captured by

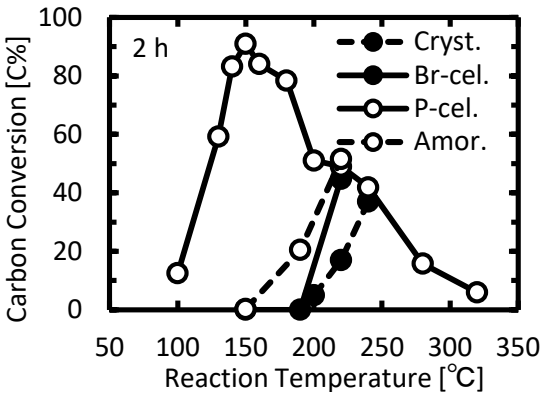

Fig. 7 Carbon conversion of P-cellulose into reducing sugar after hydrothermal treatment (on the basis of raw materials)

[EMIM]P too strongly to be separated through the reaction. However, considering the fact that reducing sugar was produced from P-cellulose by 90 C-% through the treatment, polymerization was unlikely to occur. When cellobiose and cellopentaose were analyzed by the DNS method, only cellobiose showed the output for each glucose unit while cellopentaose showed only 13 %. That means the product through the treatment on P-cellulose was mostly glucose or cellobiose, and the polymerization of them did not occur. Consequently, the valid explanation would be that glucose was captured by [EMIM]P. Then, there is a need of the explanation for the difference between P-cellulose and glucose that 90 C-% of P-cellulose was converted into reducing sugar while only 15.2 C-% of glucose was detected. For that, another experiment was conducted according to the same method using several types of samples consist of several different numbers of saccharide unit; glucose, cellobiose, fructo-oligosaccharide. The experiments without pretreatment were conducted according to the same method as well using 0.1 g of sample, 1.0 g of [EMIM]P and 8.9 g of pure water as samples. The result is shown in **Figure 8**. For the virgin samples, the result was reasonable with the yield increasing as molecular weight decreased. For the pretreated samples, on the other hand, the yield increased with molecular weight. Probably, this was because the samples were captured too strongly by [EMIM]P for the smaller molecular samples and they could not be extracted as reducing sugar anymore after the hydrothermal treatment. This two-step treatment method seems to be suitable for the bigger molecular samples such as cellulose. There is another need of the explanation for higher yield of reducing sugar from P-cellulose than virgin glucose. The first possible explanation is, some cellulose remained unsaccharified and captured by [EMIM]P, which lowered the capture ability of ionic liquid. However, this deactivation was unlikely to occur because excessive amount of [EMIM]P was used against crystalline cellulose in this reaction. The second possible explanation is, the capturing ability of [EMIM]P was lowered through the pretreatment. In this case, whether [EMIM]P still has the same performance when it was recycled would be a problem. Next, the hydrothermal degradation of P-cellulose was conducted for different reaction time at 150 ℃. The result is shown in **Figure 9**. The yield increased with reaction time. To obtain reducing sugar by more than 90 C-%, the reaction time was required to be longer than 120 min. However, this reaction time is much shorter comparing with the enzymatic saccharification process.

2.7 Recovery of Reducing Sugar and Ionic Liquid

Ionic liquid was now revealed to be effective on the saccharification of cellulose, however, it is very expensive reagent and the recovery of ionic liquid is indispensable to make the process feasible. As shown in **Figure 3**, because P-cellulose-deposit trapped much [EMIM]P, it is better to recover ionic liquid all together after converting P-cellulose to reducing sugar by the hydrothermal degradation. For the recovery, the products of the hydrothermal treatment on P-cellulose was dried once to obtain the mixture of reducing sugar, unsaccharified cellulose and ionic liquid as residue, and then 20 mL of ethanol was added. This is because only ionic liquid dissolves into ethanol while the other components do not. The product was then filtered by suction, and the solution (which is supposed to contain ionic liquid and ethanol) was heated again to evaporate ethanol for the recovery of ionic liquid. The residue was mixed with pure water for the recovery of unsaccharified cellulose as residue and reducing sugar as solute by filtration, and then recovered components were dried in vacuo and analyzed by CHNS elemental analysis to determine the components. From the analyses, the water-soluble component was clarified to consist of mostly reducing sugar and a little amount of ionic liquid. The relationship between the recovery rate of

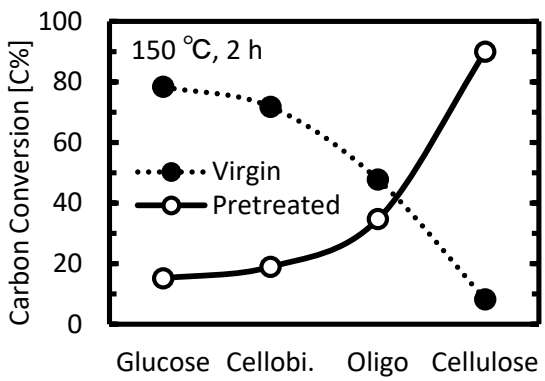

Fig. 8 Carbon conversion of different types of materials into reducing sugar after hydrothermal treatment

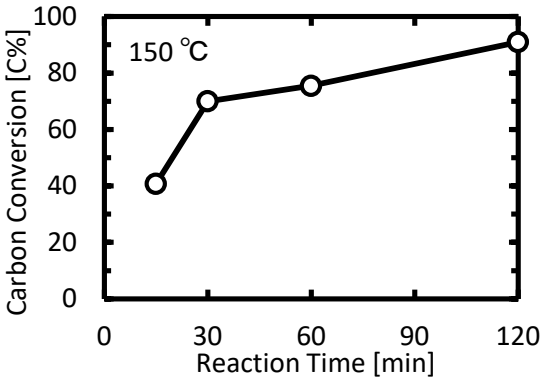

Fig. 9 Carbon conversion of P-cellulose into reducing sugar after hydrothermal treatment (on the basis of raw materials)

reducing sugar or the loss of [EMIM]P as deposit and the reaction temperature is shown in **Figure 10** with the carbon conversion into reducing sugar. The yields of ionic liquid and reducing sugar reached the highest at the reaction of 150 ℃, with the loss of ionic liquid reaching down to 1.7 wt%. Considering the value of ionic liquid, 1.7 % of loss is not small enough. However, the developed method would be only a part of whole process and further conversion of reducing sugar would help the recovery of ionic liquid, unless it inhibits the following conversions. Furthermore, the developed method which enabled the high conversion of cellulose into reducing sugar without using enzymes under mild conditions such as 2 h at 150 ℃ of the reaction might be a guide for the development of the cellulose conversion process.

Conclusions

An instructive conversion method of biomass into valuable chemical, reducing sugar, was developed for the utilization of biomass as an alternative resource to fossil resources. Ionic liquid was revealed to be an effective reagent on the production of reducing sugar from crystalline cellulose, decreasing its crystallinity. Comparing two types of ionic liquid, [EMIM]P was more effective than [EMIM]Br on the production from either samples used, crystalline cellulose and cedar. The conversion of cedar, however, was concluded as unpreferable because of the extraction of lignin by ionic liquid which requires a difficult separation. For the conversion of cellulose into reducing sugar, some experimental methods were examined for the enhancement of the yield, and significantly effective method was determined to be the hydrothermal degradation at 150 ℃ for 2 h after the pretreatment with [EMIM]P at 150 ℃ for 1 h, with the yield reaching 90 C-%. The recovery of the product and the reagent was also examined after the conversion of crystalline cellulose, and they were recovered, respectively, by 72.8% and 98.3 %. The recovery rate of ionic liquid ([EMIM]P) by 98.3 % was not high enough because of its value, however, the higher recovery rate might be achieved if the process was followed by the further conversion of the product.

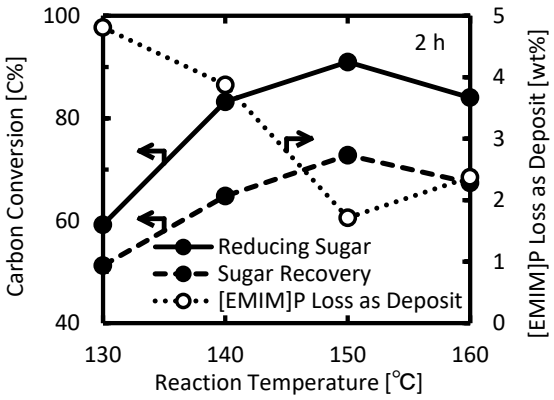

Fig. 10 Sugar recovery and loss of [EMIM]P after hydrothermal treatment (on the basis of raw materials)

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